I. INTRODUCTION

1. Principles and rational

Penicillium marneffei (*P. marneffei*) is a thermal dimorphic pathogenic fungus that causes penicilliosis marneffei, a disseminated fungal disease among immunocompetent and immunocompromised hosts, especially in acquired immunodeficiency syndrome (AIDS) patients living in southeast Asia and southern part of China. There are also a growing number of reported cases in western countries, typically in tourists returning from the endemic zone. Clearly, with the advent of the AIDS pandemic, the incidence of *P. marneffei* infection has increased and has become the third most common opportunistic infection in AIDS victims, after tuberculosis and cryptococcal meningitis (Supparatpinyo et al, 1994).

On laboratory media at 25 °C, *P. marneffei* grows as a mold form and produces red to deep red, diffusable pigment, whereas it converts to a yeast-like form at 37 °C *in vitro* or *in vivo*, displaying spherical or elliptical cells measuring 2-3 by 2-6.5 μm, which multiply by fission. The diagnosis of penicilliosis marneffei can be troublesome because its clinical manifestations mimic those of tuberculosis, *Pneumocystis carinii* pneumonia, histoplasmosis and some other mycotic infections. Furthermore, the fission yeast cells of *P. marneffei* in clinical specimens can be confused with those of nonbudding *Cryptococcus neoformans* (*C. neoformans*) and *Histoplasma capsulatum* (*H. capsulatum*) cells (Duong et al, 1996). An early and accurate diagnosis could help clinicians to give the proper treatment to decrease a mortality rate, both in normal and immunocompromised hosts.

Fungal culture for species identification is rather time-consuming. A number of presumptive diagnostic methods have been developed including serology and microscopic examinations of bone marrow aspirates, also skin and lymph node biopsy. Previously, there were several reports of antibody detection for penicilliosis based on immunodiffusion (Sekhon et al, 1982; Imwidthaya et al, 1997) or

immunofluorescent (Kaufman et al, 1995) tests with either crude antigen preparation or whole fungal cells. The tests which were developed with the crude antigen preparation or the whole fungal cells are limitations in both sensitivity and specificity. However, specific antigens of *P. marneffei*, which could be recognized by IgG antibodies from penicilliosis serum specimens were identified by Western blot assays (Chongtrakool et al, 1997; Vanittanakom et al, 1997A; Jeavons et al, 1998). The Western blot analysis revealed that the specific antigens of *P. marneffei* were recognized with individual penicilliosis serum. Recently, the recombinant Mp1p antigen, an abundant antigenic cell wall mannoprotein which was expressed from the *MP1* gene of *P. marneffei,* was used to detect antibody in individual serum from penicilliosis patients by enzyme-linked immunosorbent assay (ELISA) method. The recombinant Mp1p antigen was also used for production of the specific antibody in rabbit. The specific antibody against the recombinant Mp1p was used to detect antigen in serum samples from penicilliosis patients by ELISA method. The results revealed that the recombinant Mp1p protein was specific for host humoral immunity representing a good cell surface target of *P. marneffei,* and the ELISA which was developed with this protein offered significantly improved sensitivity and specificity for the serodiagnosis of penicilliosis marneffei (Cao et al, 1998B; Cao et al, 1999). Several studies also confirmed that *P. marneffei* infection triggered a humoral immune response (Kaufman et al, 1996; Imwidthaya et al, 1997; Verweij et al, 1997; Desakorn et al, 1999; Trewatcharegon et al, 2000). Although, some serodiagnostic tests were developed, no effective test is available for detection of an early or asymptomatic infection.

Several protein antigens of *P. marneffei* were identified from secreted and cytoplasmic antigen preparations. A 38-kilodalton (kDa) antigen isolated from mycelial culture filtrate has been shown to be recognized with a large portion of *P. marneffei*-infected HIV positive patients' sera. However, a small number of asymtomatic HIV-seropositive patients and HIV-seropositive patients with other fungal infections were found to be positive by using an immunoblot assay (Chongtrakool et al, 1997). Vanittanakom and colleagues reported the immunoblot reactivities of sera derived from 33 AIDS patients infected with *P. marneffei* against secreted yeast phase antigens (Vanittanakom et al, 1997A). The results revealed that

the major immunogenic proteins had molecular masses of 50, 54, 88 and 200 kDa. However, none of these proteins has been purified or characterized because the purification of protein from crude extract was difficult and troublesome. For instance, the 54-kDa and 88-kDa proteins could be only partially purified from crude culture filtrate using isoelectric focusing and preparative gel electrophoresis (Poolsri, 1999). Later, Jeavons and colleagues could purify a specific protein of *P. marneffei*, which has a molecular mass of 61 kDa by liquid isoelectric focusing (Jeavons et al, 1998). N-terminal amino acid sequencing result revealed that this protein had a strong homology (87% identity) with the *Escherichia coli* (*E. coli*) catalase.

At molecular levels of *P. marneffei* antigens, a few investigations have been reported. Cao and colleagues reported that the *MP1* gene encoded antigenic cell wall mannoprotein, which was a Mp1p protein (Cao et al, 1998A). The Mp1p protein was used for the detection of antibody against Mp1p and for the production of the specific rabbit anti-Mp1p, which was used for the detection of antigen in serum samples of *P. marneffei*-infected patients by using the ELISA assay. Sera from healthy blood donors, patients with typhoid fever, and patients with tuberculosis were used as controls. The result revealed that the Mp1p is a specific antigen of *P. marneffei* (Cao et al, 1998B; Cao et al, 1999). However, the rabbit anti-Mp1p serum could not react specifically with protein antigens of *P. marneffei* Thai isolates (Unpublished data). Therefore, the specific antigens of *P. marneffei* isolated from Thai patients have to be identified in order to develop a test for detection of the specific antibody to *P. marneffei*. Recently, a cDNA library of the yeast phase of *P. marneffei* has been successfully constructed and screened by using an antibodies screening technique. Several different genes encoding immunogenic proteins were cloned (Pongpom, 2004). The characterization of five clones revealed the encoding regions of putative catalase peroxidase, 30-kDa heat shock protein (HSP30), and three unknown genes. In this study, two of those genes expressing *P. marneffei* antigens were used for the production of the recombinant proteins.

The goal of this study was to clone two genes of interest encoding *P. marneffei* proteins into an expression vector, pGEX-4T-1, by using molecular cloning technique. The recombinant proteins were produced in bacteria and purified using affinity

chromatography. Then the purified proteins were used to detect specific antibody in sera of *P.marneffei*-infected AIDS patients by Western blot assay.

2. Objectives

In this study, the unknown gene, *P6* cDNA and *P23* cDNA encoding putative HSP30 were used for generating the recombinant proteins. To achieve the goals, it was necessary to:-

1. To generate plasmid DNA constructs encoding *P. marneffei* proteins, which are selected from the cDNA cloned genes
2. To express the recombinant *P. marneffei*-fusion proteins in bacteria
3. To purify the recombinant proteins by affinity chromatography
4. To evaluate the immunoreactivities of recombinant proteins by using Western blot analysis

3. Education/Application advantages

1. The specific recombinant antigens of this fungus could be used as diagnosis markers on development of a powerful serological test to detect early stage or asymptomatic *P. marneffei* infection.

2. The developed test might be used for the epidemiological study in determining of the previous exposure to this fungus among people living in the endemic area.

3. The immunogenic portion of the agent could be determined and used as a vaccine candidate or drug target.